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Cigarette smoke impairs neutrophil respiratory burst activation by aldehyde-induced thiol modifications

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Abstract

Exposure to airborne pollutants such as tobacco smoke is associated with increased activation of inflammatory-immune processes and is thought to contribute to the incidence of respiratory tract disease. We hypothesized that cigarette smoke (CS) could synergize with activated inflammatory/immune cells to cause oxidative injury or result in the formation of unique reactive oxidants. Isolated human neutrophils were exposed to gas-phase CS, and the production of nitrating and chlorinating oxidants following neutrophil stimulation was monitored using the substrate 4-hydroxyphenylacetate (HPA). Stimulation of neutrophils in the presence of CS resulted in a reduced oxidation and chlorination of HPA, suggesting inhibition of NADPH oxidase or myeloperoxidase (MPO), the two major enzymes involved in inflammatory oxidant formation. Peroxidase assays demonstrated that neutrophil MPO activity was not significantly affected after CS-exposure, leaving the NADPH oxidase as a likely target. The inhibition of neutrophil oxidant formation was found to coincide with depletion of cellular GSH, and a similar modification of critical cysteine residues, such as those in NADPH oxidase components, might be involved in reduced respiratory burst activity. As α,β-unsaturated aldehydes such as acrolein have been implicated in thiol modifications by CS, we exposed neutrophils to acrolein prior to stimulation, and observed inhibition of NADPH oxidase activation in relation to GSH depletion. Additionally, translocation of the cytosolic components of NADPH oxidase to the membrane, a necessary requirement for enzyme activation, was inhibited. Protein adducts of acrolein (or related aldehydes) could be detected in several neutrophil proteins, including NADPH oxidase components, following neutrophil exposure to either CS or acrolein. Alterations in neutrophil function by exposure to (environmental) tobacco smoke may affect inflammatory/infectious conditions and thereby contribute to tobacco-related disease.

Keywords: Aldehyde-induced thiol modifications; Cigarette smoke; Neutrophil respiratory burst activation

1. Introduction

Cigarette smoking and exposure to environmental tobacco smoke have been identified as

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major risk factors for both respiratory and cardiovascular diseases. Cigarette smoke (CS) contains an abundance of reactive chemicals and particles that can directly injure cells, cause mutagenesis, or result in the activation of inflammatory-immune processes. One common feature of cigarette smokers is the presence of elevated numbers of inflammatory cells in the respiratory tract and generally increased susceptibility to activation, and the contribution of CS to respiratory tract disease is most likely determined by interactions between direct CS-induced effects and (chronic) inflammatory-immune processes. Despite our increased understanding of the relationships between CS exposure and respiratory tract inflammatory processes, the exact molecular mechanisms by which smoking may contribute to typical CS-related diseases, such as chronic bronchitis, emphysema or lung cancer, are still incompletely understood. More specifically, despite the presence of increased inflammation in smokers, CS exposure can directly affect inflammatory-immune processes, and may thereby contribute to reported increases in host susceptibility to respiratory infections (e.g. Johnson et al., 1990). In addition, CS exposure has also been linked to increases in respiratory illnesses and symptoms of asthma and chronic bronchitis, especially in children (e.g. Witschi et al., 1997). The biochemical basis for these associations is, however, still largely unclear.

One common aspect of CS-related respiratory diseases is the presence of oxidative stress, as observed in such cases as emphysema, chronic bronchitis or asthma (Rahman and MacNee, 1996; Floreani and Rennard, 1999; Seagrave, 2000). CS itself contains an abundance of oxidants and nitrogen oxides that might be directly responsible for such oxidative lung injury, but inflammatory-immune processes that are commonly activated in these cases are likely to contribute to this. Depending on the participating cell types, oxidative stress during inflammation involves the activation of phagocyte peroxidases such as the neutrophil enzyme myeloperoxidase (MPO) or eosinophil peroxidase (Hazen and Heinecke, 1997; Foreman et al., 1999; Wu et al., 2000; van der Vliet et al., 2000). These enzymes have recently been found to be involved in formation of reactive nitrogen intermediates from nitric oxide ($NO^{\bullet}$) and/or their metabolites (Eiserich et al., 1998; van der Vliet et al., 1999). Since nitrogen oxides [primarily $NO^{\bullet}$ but also other reactive nitrogen intermediates such as nitrogen dioxide ($NO_2^{\bullet}$) and peroxynitrous acid (ONOOH)] are abundant in CS (Norman and Keith, 1965; Pryor and Stone, 1993), such interactions with phagocyte peroxidases could give rise to heightened nitrosative stress during CS exposure, especially in combination with activated inflammatory/immune processes in the lung. Moreover, nitrogen oxides in CS itself can account for much of the oxidative stress associated with CS exposure (Eiserich et al., 1994, 1997).

CS can, however, also impair phagocyte or macrophage function (Drost et al., 1992; Selby et al., 1992; Herlihy et al., 1995; Braun et al., 1998), and various components of CS have been demonstated to be capable of inhibiting phagocyte respiratory burst (NADPH oxidase) activation. Several authors have observed that $NO^{\bullet}$ or some of its metabolites can inactivate NADPH oxidase activation, presumably by a direct action on this enzyme system (Clancy et al., 1992; Park, 1996; Fujii et al., 1997; Rodenas et al., 1998). Similarly, reactive aldehydes that are abundant in CS can also inhibit NADPH oxidase activation (Witz et al., 1987; Dianzani et al., 1996; Siems et al., 1997). In the present investigation, the potential effects of CS on inflammatory oxidant formation were studied in isolated human neutrophils, by analysis of production of oxidizing, chlorinating and nitrating intermediates that reflect the formation of either CS-derived or inflammatory oxidants. It was found that formation of chlorinating oxidants by stimulated neutrophils was dramatically reduced following exposure to CS, suggesting inhibition of neutrophil oxidant generation. Moreover, neutrophil stimulation did not enhance the formation of nitrating intermediates by CS. Inhibition of the neutrophil respiratory burst by CS was found to coincide with depletion of cellular GSH and could be mimicked by acrolein, a major α,β-unsaturated aldehyde in CS. The inhibitory effects of acrolein may be due to alteration of cellular redox status by depletion of GSH

or to direct alkylation of critical cell constituents, such as components of the NADPH oxidase complex itself. Diminished phagocyte function by CS-related reactive aldehydes may affect inflammatory/infectious conditions and thereby contribute to CS-related disease.

2. Materials and methods

2.1. Neutrophil isolation and exposure

Polymorphonuclear neutrophils were isolated from venous blood, obtained by venipuncture from healthy non-smoking volunteers, using Lymphocyte Separation Medium (ICN Biochemicals, Aurora, OH) centrifugation and dextran sedimentation, as described previously (e.g. Eiserich et al., 1998). This usually resulted in > 95% pure neutrophils with >98% viability. Neutrophils were suspended at 2×10^6 cells/ml in PBS containing 1.2 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 5 mM glucose (pH 7.4), and 3 ml of this suspension were placed into a polysterene T25 flask. Fresh smoke from a University of Kentucky 2R1 research cigarette was then introduced to the flask (5–20 ml) and the flask was sealed tightly. In some cases, neutrophils were also stimulated with 50 ng/ml of phorbol myristate acetate (PMA; RBI, Natick, MA) immediately prior to CS exposure. Similar exposures were performed with pure acrolein (Aldrich, Milwaukee, WI), the major α,β-unsaturated aldehyde in CS, at concentrations from 1 to 50 μM (0.5–25 fmol/cell). Following incubations for up to 30 min at 37°C, cells and incubation media were collected separately for analysis of cellular GSH, modifications of neutrophil proteins, nitrite (as an index of CS exposure), and oxidant formation.

2.2. HPLC analysis of aromatic nitration, chlorination, and oxidation

To determine the formation of neutrophil- or CS-derived oxidants, neutrophil incubations were carried out in the presence of 1 mM 4-hydroxyphenylacetate (HPA, Sigma), and oxidative modifications of this detector molecule were determined by HPLC as described previously (van der Vliet et al., 1997). Previous studies indicated that neutrophil activation causes the formation of both 3,3′-diHPA and 3-chloro-HPA, products of oxidation and chlorination, respectively. Conversely, exposure of HPA to CS results in both oxidation and nitration (formation of 3-nitro-HPA), but not chlorination. Thus, this allows us to distinguish the generation of either neutrophil- or CS-derived oxidants.

2.3. Neutrophil MPO activity

Myeloperoxidase (MPO) activity of neutrophils was determined spectrophotometrically using guaiacol (Sigma) as a substrate (van der Vliet et al., 2000). Neutrophils were lysed in the presence of 0.1% Triton to recover cellular MPO. MPO activity was expressed relative to the neutrophil protein content, which was determined using the Bradford-based Bio-Rad protein assay.

2.4. Analysis of cellular GSH

Neutrophils were lysed in solubilization buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 2 mM Na_3VO_4, 0.1% Triton, 10% glycerol, 1 mM PMSF, 10 μg/ml of aprotinin, and 10 μg/ml of leupeptin, pH 7.5) following exposure to either CS or acrolein, and cellular GSH was analysed in cell lysates after derivatization with monobromobimane (Calbiochem, San Diego, CA) and HPLC analysis with fluorescence detection (van der Vliet et al., 1998).

2.5. Analysis of NADPH oxidase activation

Activation of the neutrophil respiratory burst (NADPH oxidase) was analyzed by measuring SOD-inhibitable $O_2^{\cdot -}$ production using ferricytochrome c reduction after neutrophil stimulation with 100 ng/ml of fMLP (McCord and Fridovich, 1969). One aspect of activation of NADPH oxidase is the translocation of cytosolic NADPH oxidase components to the plasma membrane (Clark et al., 1990), and the effects of

acrolein on this pathway were studied. To this end, neutrophils (2×10^7 cells/ml) in suspension were exposed to 200 μM acrolein (10 fmol/cell) and PMA, and subsequently lysed in 1 ml of relaxation buffer (100 mM KCl, 3 mM NaCl, 3.5 mM $MgCl_2$, 1.25 mM EGTA, 10 mM piperazine diethane sulfonic acid, pH 7.3) by sonication (5 s). Unbroken cells and nuclei were removed by a 5 min centrifugation at $250 \times g$, and the resulting supernatant was centrifuged for 20 min at $115\,000 \times g$. The resulting pellet was washed in relaxation buffer, centrifuged again and finally suspended in 0.3 ml of relaxation buffer by 3 s sonication. The presence of NADPH oxidase subunits ($p47^{phox}$ and $p67^{phox}$) in either fraction was determined by mixing with 2 × Laemmli sample buffer, separation by 10% SDS–PAGE and Western blot analysis using monoclonal antibodies (Transduction Laboratories, Lexington, KY).

2.6. Determination of protein–aldehyde adducts

Two procedures were used to detect protein–aldehyde adducts in neutrophil lysates after exposure to either CS or acrolein. The first involved derivatization of cell lysates with dinitrophenylhydrazine (DNPH), after which proteins were subjected to SDS–PAGE and Western blot analysis using an α-DNP-antibody (OxyBlot® Oxidized Protein Detection Kit, Intergen). To detect the potential formation of aldehyde adducts in specific NADPH oxidase components, these components were immunoprecipitated from cell lysates using protein A-sepharose and subsequently subjected to DNPH derivatization and analysis. As an alternative approach, protein acrolein adducts were detected by separation of neutrophil lysates (mixed with 2 × Laemmli buffer) by 10% SDS–PAGE and Western Blot analysis using mAb5F6, an antibody against N^{ε}-(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine), kindly provided by Dr. K. Uchida, Nagoya University, Japan (e.g. Uchida et al., 1998). Antibodies were detected using HRP-conjugated secondary antibodies and visualized by ECL^{plus} (Amersham, Piscataway, NJ) using a Phosphorimager (Storm 860, Molecular Dynamics).

3. Results

3.1. Cigarette smoke inhibits the formation of chlorinating oxidants by stimulated neutrophils

Stimulation of human neutrophils with 100 ng/ml of PMA resulted in activation of the respiratory burst, as demonstrated by the oxidation and chlorination of the aromatic substrate HPA (Fig. 1). Some inadvertent activation occurred in the absence of PMA, presumably because of neutrophil adhesion to the culture flask. Both oxidative modifications could be prevented by the MPO-inhibitor aminobenzoic acid hydrazide (ABAH; Aldrich), indicating a critical involvement of MPO. Consistent with earlier findings, stimulation of neutrophils did not result in signifi-

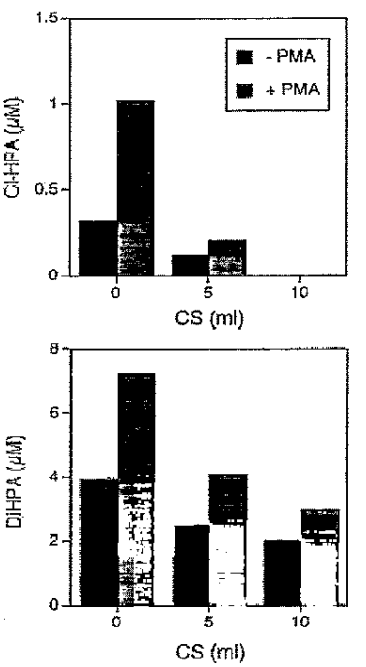


Fig. 1. CS exposure inhibits formation of neutrophil-derived oxidants. Neutrophils (2×10^6/ml) were placed in a T-25 culture flask, and a puff of fresh CS (5–10 ml) was introduced into the flask. Where indicated, neutrophils were also activated with PMA immediately prior to CS exposure. The formation of neutrophil oxidants was assessed by determination of chlorination and oxidation of the target molecule HPA, to form Cl-HPA (top) and diHPA (bottom), respectively.

cant nitration of HPA. Exposure of HPA to 5–60 ml of CS in the absence of neutrophils resulted in nitration and oxidation (but not chlorination) of HPA, reflecting the oxidative potential of CS itself. Both products increased linearly with the dose of CS added; for instance, exposure of 1 mM HPA to 60 ml of gas-phase CS for 30 min yielded 1.3 μM NO_2-HPA and 0.4 μM diHPA.

Both basal and PMA-mediated neutrophil-dependent HPA oxidation and chlorination were dramatically inhibited by CS (Fig. 1). At doses >10 ml of CS, HPA chlorination was undetectable, and formation of diHPA reduced to levels equivalent to that by CS alone. Additionally, HPA nitration was observed to a degree similar to that by CS alone. These results indicate that CS markedly inhibits neutrophil oxidant generation, and we observed no evidence of synergistic oxidation or nitration by stimulated neutrophils and CS.

3.2. Effect of CS exposure on neutrophil MPO.

Exposure of neutrophils to up to 20 ml CS did not affect mitochondrial respiration of neutrophils, as determined using Alamar Blue® (Alamar Bioscience, Sacramento, CA), suggesting that viability was not significantly decreased. Moreover, the CS exposure did not cause detachment of neutrophils or noticable alterations in cell morphology (not shown). The formation of neutrophil oxidants requires both activation of NADPH oxidase as well as the granule enzyme myeloperoxidase (MPO), and CS could affect the activity of either enzyme. Measurement of neutrophil MPO activity showed no significant change after CS exposure (2.4 ± 0.3 U/mg of protein in untreated cells and 2.7 ± 0.6 and 2.2 ± 0.3 U/mg of protein in neutrophils exposed to 5 and 10 ml CS, respectively). To verify the activity of neutrophil MPO after CS exposure, 100 μM H_2O_2 was added to the incubations after neutrophil exposure to CS, and MPO-dependent oxidation of HPA was monitored. As shown in Fig. 2, additional HPA oxidation was induced by H_2O_2 in both stimulated and unstimulated cells, and this was partially inhibited after neutrophil exposure to 10 ml of CS. This inhibition was most

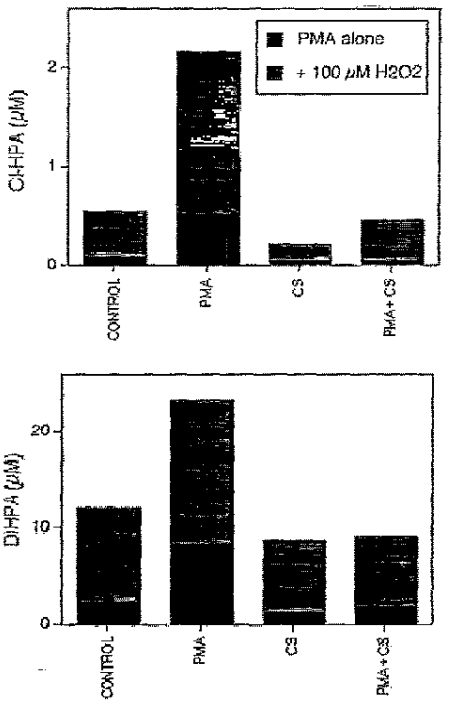


Fig. 2. Effect of CS exposure on neutrophil MPO activity. Neutrophils were exposed to CS and/or PMA in the presence of 1 mM HPA, as in Fig. 1. Following these exposures, MPO-dependent oxidation of HPA was assessed after addition of 100 μM H_2O_2, and additional HPA chlorination (top) and oxidation (bottom) was measured. Black bars: CS or PMA alone; shaded bars: additional chlorination/oxidation by addition of H_2O_2.

pronounced in PMA-stimulated cells, suggesting that HPA oxidation and chlorination may depend on MPO degranulation, which might be inhibited by CS exposure. Nevertheless, our findings indicate that MPO is not a major target for CS and that other factors, such as NADPH oxidase itself, may be targeted by CS components.

3.3. Depletion of cellular GSH by CS or CS-derived acrolein

Exposure of neutrophils to CS resulted in rapid depletion of cellular GSH, which occurred in the first 5 min after exposure. The degree of GSH depletion by CS correlated with the inhibition of neutrophil-dependent HPA chlorination, and cel-

lular GSH was nearly completely depleted after exposure to > 10 ml of CS (Fig. 3), under which conditions, the formation of neutrophil oxidants was almost completely inhibited. CS-induced GSH or thiol depletion has often been linked to the presence of α,β-unsaturated aldehydes (acrolein, crotonaldehyde), and previous studies have suggested that unsaturated aldehydes present in CS may be primarily responsible for CS reactions with thiols and GSH (e.g. Reznick et al., 1992). Therefore, we performed similar experiments with acrolein, the major CS-related α,β-unsaturated aldehyde, and found that acrolein at concentrations between 0.1 and 10 μM also readily depletes GSH from neutrophils.

3.4. Inhibition of NADPH oxidase by acrolein

Similar to CS, acrolein was also capable of diminishing neutrophil-dependent oxidation and chlorination of HPA. Treatment of neutrophils with > 10 μM acrolein (> 5 fmol/cell) completely inhibited PMA-dependent HPA chlorination and inhibited diHPA formation by > 80%. To determine more directly the inhibition of NADPH oxidase activation, respiratory burst activation was measured using cytochrome c reduction, and acrolein (0.1–10 μM) was found to inhibit fMLP-stimulated activation of the neutrophil respiratory burst (Fig. 4A). Activation of NADPH oxidase requires translocation of the cytosolic proteins $p47^{phox}$ and $p67^{phox}$ to the plasma membrane, to

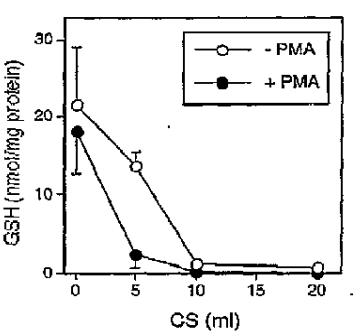


Fig. 3. CS and CS-related aldehydes rapidly deplete cellular GSH. Neutrophils were exposed to gas-phase CS for 10 min, and cellular GSH was measured by HPLC after derivatization with monobromobimane.

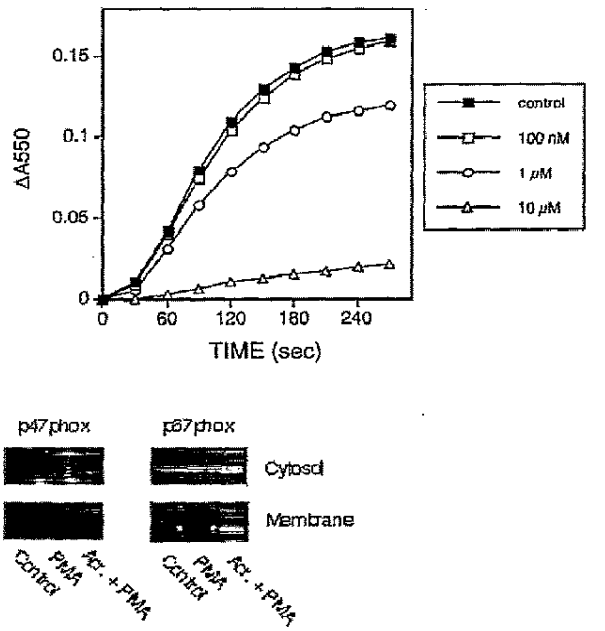
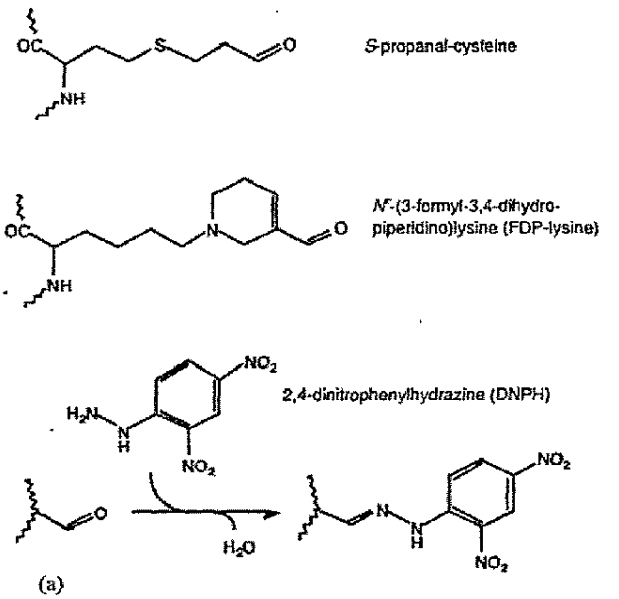


Fig. 4. Acrolein inhibits activation of NADPH oxidase in neutrophils. (A) Neutrophils were suspended at 2×10^6 cells/ml, and treated with 0.1–10 μM acrolein for 10 min, after which the respiratory burst activity was measured after stimulation with fMLP or PMA by ferricytochrome c reduction (expressed as ΔA_{550}). (B) Acrolein-treated neutrophils were stimulated with PMA and subsequently lysed, and membrane and cytosolic fractions were prepared by ultracentrifugation. Translocation of cytosolic NADPH oxidase subunits to the plasma membrane was then assessed by SDS–PAGE and Western blot analysis of cytosolic and membrane fractions.

associate with the membrane-associated components of this enzyme complex. As shown in Fig. 4B, exposure of neutrophils to acrolein (10 fmol/cell) prevented PMA-indcued translocation of these subunits to the membrane compartment, thus illustrating that acrolein can inhibit pathways that induce assembly of this enzyme complex.

3.5. Determination of protein-acrolein adducts in neutrophils

Exposure to CS and/or unsaturated aldehydes such as acrolein can result in the formation of protein carbonyls (e.g. Reznick et al., 1992), which is often considered a measure of protein oxidation. However, α,β-unsaturated aldehydes



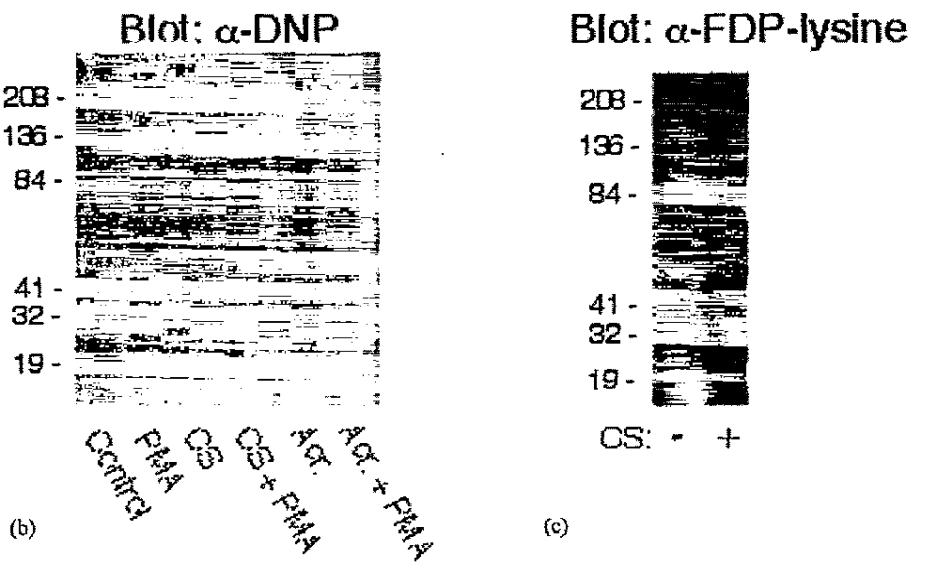


Fig. 5. Formation of protein–aldehyde adducts in neutrophils by CS and acrolein exposure. Neutrophils were exposed to either CS or acrolein in the presence or absence of PMA (as in Figs. 1 and 4), and the formation of protein–aldehyde adducts was determined by SDS–PAGE and Western blot analysis. (A) Schematic representation of acrolein adducts with cysteine or lysine residues, and derivatization with DNPH. (B) Neutrophil proteins were derivatized with DNPH and subjected to SDS–PAGE and Western blot analysis of DNP-labeled proteins. (C) Neutrophil proteins were subjected to SDS–PAGE and Western blot analysis of the protein–acrolein adduct FDP–lysine.

such as acrolein form similar protein carbonyls by Michael addition to protein cysteine residues (Esterbauer et al., 1975). Derivatization of neutrophil proteins with DNPH, followed by SDS–PAGE/Western blot analysis, revealed the presence of protein carbonyls in several neutrophil proteins, after exposure to either gas-phase CS or acrolein (Fig. 5). Formation of protein–acrolein adducts in CS-exposed neutrophils was demonstrated more directly using an antibody against FDP-lysine, a specific product from acrolein addition to lysine residues. It is feasible that such covalent protein modifications might be involved in alterations in neutrophil function. Additionally, GSH and protein thiol residues are thought to be major targets for CS-derived acrolein and related unsaturated aldehydes, and NADPH oxidase subunits contain cysteine residues that appear to be critical for assembly and activation of this enzyme complex (e.g. Babior 1999). Hence, we investigated whether CS-derived acrolein could alkylate NADPH oxidase components directly. Indeed, immunoprecipitation experiments demonstrated the presence of carbonyl adducts within $p47^{phox}$, a cytosolic subunit of NADPH oxidase, following neutrophil exposure to either CS or to acrolein (Fig. 6), suggesting addition of acrolein or related aldehydes to this subunit. Such covalent modification of $p47^{phox}$ could conceivably contribute to the observed inhibition of respiratory burst activation.

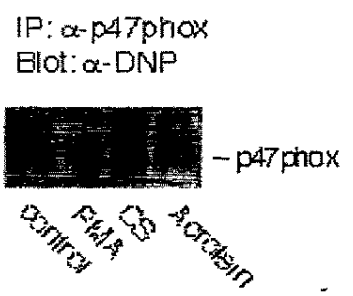


Fig. 6. Formation of acrolein adducts in the NADPH oxidase protein $p47^{phox}$. Neutrophils were exposed to CS or acrolein, and $p47^{phox}$ was immunoprecipitated from neutrophil lysates using a polyclonal antibody against $p47^{phox}$. Following derivatization of the immune complex with DNPH, the precipitated proteins were analyzed by SDS–PAGE and Western blotting with an α-DNP-antibody.

4. Discussion

The major findings of the present study are that direct exposure of polymorphonuclear neutrophils to gas-phase CS does not result in increased nitrosative stress, but in fact impairs neutrophil function, as indicated by inhibited activation of the neutrophil respiratory burst. We present evidence that the major α,β-unsaturated aldehydes in CS (acrolein, crotonaldehyde, etc.) may be responsible for this, either by depleting cellular GSH and thereby changing cellular redox status, or by direct alkylation of cysteine residues in proteins involved in NADPH oxidase activation (including components of NADPH oxidase itself). Such CS-induced changes in phagocyte function could be involved in the immunosuppressive effects of CS.

In pioneering experiments over 30 years ago, Green demonstrated that bacterial phagocytosis by alveolar macrophages is inhibited by CS, and that GSH or cysteine could abolish this effect of CS (Green, 1968). Moreover, the immunosuppressive effects and/or cell injury by CS appear to be due primarily to the vapor phase of CS and were found to be related to SH reactivity (Leuchtenberger et al., 1974). The vapor (gas) phase of CS contains numerous reactive chemicals, but is especially abundant in volatile aldehydes, including α,β-unsaturated aldehydes such as acrolein and crotonaldehyde (e.g. Eiserich et al., 1997), that are reactive with GSH or other biological SH groups (Esterbauer et al., 1975). Indeed, many cellular effects of CS appear to be related to changes in SH redox status, and reactive volatile aldehydes, such as acrolein or crotonaldehyde, may be primarily responsible for such effects (Rahman and MacNee, 1996; Meacher and Menzel, 1999). Indeed, the inhibitory effects of CS on the neutrophil respiratory burst were closely related to depletion of cellular GSH and could be mimicked by acrolein (at doses similar to those in CS), suggesting that acrolein or related aldehydes may be responsible for the inhibitory effects of CS.

Several previous investigators have demonstrated that acrolein or similar aldehydes can inhibit NADPH oxidase activation in neutrophils or macrophages (Witz et al., 1987; Siems et al.,

1997), and it was suggested that reaction with soluble or membrane SH groups are involved in this. Using various immunological procedures, we confirmed that neutrophil exposure to CS or acrolein results in the formation of various aldehyde and/or acrolein adducts. We attempted to characterize further the specific targets for CS-derived acrolein and considered that components of the NADPH oxidase itself could be directly alkylated. Indeed, our results suggest that $p47^{phox}$ might represent such a target. This cytosolic factor of NADPH oxidase contains three free cysteine residues that appear to be involved in enzyme activation (Inamani et al., 1998; Babior, 1999). The membrane-associated $gp91^{phox}$ has also been considered to be susceptible to redox-modification, and thus could present an additional target (e.g. Park, 1996). Efforts to detect aldehyde adducts in $gp91^{phox}$ in CS-exposed neutrophils, using immunoprecipitation strategies, were unfortunately unsuccesful. Phenylarsine oxide (PAO), a reagent that primarily targets vicinal thiols, has been shown to inhibit NADPH oxidase activation (Kutsumi et al., 1995; Le Cabec and Maridonneau-Parini, 1995) but, unlike acrolein, did not affect translocation of cytosolic NADPH oxidase components upon neutrophil stimulation (Le Cabec and Maridonneau-Parini, 1995). Other thiol reagents, such as *N*-ethylmaleimide, have been demonstrated to inhibit NADPH oxidase activation and membrane translocation of cytosolic NADPH oxidase components (Clark et al., 1990). Collectively, there are several potential targets for acrolein (or SH reactive chemicals) that mediate inhibition of NADPH oxidase activation, possibly including protein kinase C, tyrosine phosphatases, or components of the NADPH oxidase itself.

The rapid reaction of cellular GSH (which occurs within the first few minutes after acrolein exposure) could also indirectly cause cellular redox changes that affect neutrophil function. Acrolein is known to react spontaneously with GSH via the formation of a Michael adduct (Esterbauer et al., 1975), but this reaction is most likely catalyzed by cellular glutathione S-transferases. Such GSH depletion may indirectly affect the redox status of many redox-sensitive proteins and thus indirectly affect enzymatic pathways that may be involved in neutrophil activation. Indeed, several recent studies have indicated that acrolein can inhibit cell proliferation, which may be secondary to alterations in cellular redox status. Moreover, acrolein could also effect changes in the expression of growth- or stress-related genes or transcription factors, and has been shown to be capable of inhibiting the activation of the transcription factors nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) (Kehrer and Biswal, 2000). Future studies directed to identification of direct cellular targets for acrolein or related aldehydes may clarify further the potential cellular responses to this toxicant. In contrast to oxidant-induced signaling, modification of cysteine residues by acrolein or related aldehydes might be resistant to GSH reductase or thioredoxin/glutaredoxin systems, and thus induce more persistent and perhaps more dramatic cellular changes.

In summary, our results indicate that CS may directly impair neutrophil function, and the presence of volatile reactive aldehydes such as acrolein appears to be a significant factor in this respect. Indeed, acrolein has been shown to be capable of suppressing pulmonary host defense (Li and Holian, 1998), and the results presented here may provide an additional biochemical explanation for the adverse affects of smoking on chronic inflammatory diseases of the respiratory tract.

Acknowledgements

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